



Correlation between Matrix Metalloproteinase-9 (MMP-9) with Clinical Staging of Papillary Thyroid Carcinoma (PTC)

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Abstract

Background: Thyroid cancer is the most common endocrine cancer with an increasing incidence in the world. Immunohistochemistry is widely used to differentiate benign and malignant lesions in thyroid cancer through the identification of molecular markers. In this study, we aimed to examine the association between matrix metalloproteinase-9 (MMP-9) expression and its relationship with the clinical stage of PTC patients.

Methods: We recruited 26 patients with PTC in Dr. Soetomo General Hospital (Surabaya, Indonesia) in between September 2020-Agustus 2021. MMP-9 expression was examined using immunohistochemistry. Clinical staging was determined using 8th American Joint Committee on Cancer (AJCC) guideline.

Results: The number of females was higher than males (73.1% and 26.9%, respectively). There was no significant association between clinical stage and MMP-9 expression ($P = 0.16$). The results of the analysis also showed that there were no statistically significant association between T, N, and M with MMP-9 expression ($P > 0.05$ for all).

Conclusion: There was no significant association between MMP-9 expression and clinical staging of papillary thyroid carcinoma.

Keyword: Matrix metalloproteinase-9, clinical staging, papillary thyroid carcinoma, TNM system

Introduction

Thyroid cancer is the most common endocrine cancer with an increasing incidence in the world (1). The prevalence of thyroid cancer is reported as high as 2% of the total cancer cases in the world (2). In Indonesia, thyroid cancer ranks 10th of all malignancies with a prevalence of 3.8% of all malignancies (3). Thyroid cancer predominantly occurs in women with the main risk factors of having a family history of thyroid cancer and history of radiation exposure to the head and neck (4). The average thyroid cancer in men is found at the age of 65 to 69 years while in women at the age of 45 to 49 years (5). Papillary Thyroid Carcinoma (PTC) is the most common histological type of thyroid cancer, which estimated around 90% of all thyroid cancers (5).

Immunohistochemistry is widely used to differentiate benign and malignant lesions in thyroid cancer through the identification of molecular markers (6). Immunohistochemistry also has potential as a prognostic marker of thyroid cancer (7). Several markers were proposed to detect thyroid cancer, such as Hector Battifora mesothelial-1 (HBME-1), galectin-3 (GAL3), cytokeratin 19 (CK19), Cbp/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain 1 (CITED1), and thyroperoxidase (TPO). However, there is no sensitive marker for the diagnosis of thyroid cancer (6).

Matrix metalloproteinase-9 (MMP-9) is an enzyme that is part of the matrix metalloproteinase group. MMP-9 is a zinc dependent enzyme and plays a role in the degradation of the extracellular matrix (8,9). MMP-9 is associated with tumor invasion and metastasis (10,11). MMP-9 is reported to play an important role in tumor metastasis through regulation of tumor growth, angiogenesis, cell migration and invasion(8). Selemetjev et al. found a significant relationship between MMP-9 expression and clinical stage of thyroid cancer patients (12). However, there were limited number of published data about MMP-9 expression in PTC patients. This study we aimed to examine the association between MMP-9 expression and its relationship with the clinical stage of PTC patients.

Materials and Methods

Study design and clinical staging of patients

This study is an analytical observational study with a cross-sectional study design. In this study, the measurement of variables was carried out once and at a time to see the expression of MMP-9 and the clinical stage of PTC. This research was conducted at the Department of Surgery, Faculty of Medicine Universitas Airlangga/ Dr. Soetomo General Hospital (Surabaya, Indonesia). The examination of MMP-9 expression was carried out at the Anatomical Pathology Laboratory, Faculty of Medicine Universitas Airlangga/ Dr. Soetomo General Hospital (Surabaya, Indonesia).

We recruited patients with PTC in Dr. Soetomo General Hospital (Surabaya, Indonesia) in between September 2020-Agustus 2021. Diagnosis was made based on history, physical examination, and histopathological biopsy results. The exclusion criteria were

patients that have been diagnosed with other malignancies, patients who came with residual cancer, and patients with no feasible paraffin block. The clinical staging of PTC type patients is based on the was determined using 8th American Joint Committee on Cancer (AJCC) guideline.

MMP-9 expression examination

MMP-9 expression was examined using immunohistochemistry. The immunohistochemistry was performed using antibodies against MMP-9 protein. The positive result of MMP-9 staining is showed by brown color on the cytoplasm of tumor cells and a semi-quantitative score is made based on the percentage of stained cells as previously described (Vasala, 2008). Immunohistochemistry and MMP-9 expression assessment were performed by experienced pathologist.

Statistical analysis

The statistical analysis was performed using the SPSS statistical software package version 23.0 (IBM Corp., Armonk, NY, USA). Discrete variables were tested using the Chi-square test. Statistical significance was determined when the P value was less than 0.05.

Results

Subject's characteristics

In accordance with the inclusion and exclusion criteria, we included a total of 26 samples in this study. In this study the number of females was higher than males (73.1% and 26.9%, respectively). The predominant age group was under 55 years (57.7%). Age grouping was adjusted according to the need for TNM-based clinical classification of thyroid cancer based on the 8th American Joint Committee on Cancer (AJCC) stage. Based on observations, we found that T2N0M0 is the most common clinical stage. This condition means that most of the study samples received treatment after the tumor size was between 2 to 4 cm and limited to the thyroid. The subjects's characteristics is shown in Table 1.

Table 1. Subject's characteristics

Characteristic	Total (n)	Percentage (%)
Total number	26	100%
Sex		
Male	7	26.9%
Female	19	73.1%
Age group		
≤ 55 years	15	57.7%
> 55 years	11	42.3%
Staging		
T2N0M0	8	30.8%
T2N0M1	1	3.8%
T2N1bM0	2	7.7%
T2N1bM1	1	3.8%
T3aN0M0	1	3.8%
T3N0M0	1	3.8%
T3N1bM0	2	7.7%
T3N1bM1	1	3.8%
T3N1M0	1	3.8%
T4aN1aM0	1	3.8%
T4aN1bM0	1	3.8%
T4aN1M0	2	7.7%
T4bN0M0	1	3.8%
T4bN1aM1	1	3.8%
T4N0M0	1	3.8%
T4N1bM0	1	3.8%
Stage T		
T2	12	46.2%
T3	5	19.2%
T3a	1	3.8%
T4	2	7.7%
T4a	4	15.4%
T4b	2	7.7%
Stage N		
N0	13	50.0%
N1	3	11.5%
N1a	2	7.7%
N1b	8	30.8%
Stage M		
M0	22	84.6%
M1	4	15.4%

Clinical staging and MMP-9 expression of subjects

We examine the clinical staging and the expression of MMP-9 of our patients (Table 2). We found that most of our patients were in the early stages (clinical stages I and II) as many as 80.7%. Meanwhile, the advanced stage (clinical stage III and IV) was found in 19.2% patients. There were 9 patients with +1 score in this study (9/26; 34.6%), where the result showed that 5-25% of tumor cells were smeared on immunohistochemistry. Meanwhile, there were 10 patients with score +2 and +3 altogether (5 patients with +2 score and 5 patients with +3 score).

Table 2. Clinical staging and MMP-9 expression of subjects

Characteristic	Total (n)	Percentage (%)
Clinical staging		
I	16	61.5%
II	5	19.2%
III	2	7.7%
IVA	1	3.8%
IVB	2	7.7%
MMP-9 expression		
0	7	26.9%
+1	9	34.6%
+2	5	19.2%
+3	5	19.2%

Association between clinical staging and tumor status with MMP-9 expression

We examined the association between clinical staging and tumor status with MMP-9 expression (Table 3). The results of cross tabulation did not show any significant association between clinical stage and MMP-9 expression. In clinical stage I, the distribution of MMP-9 expression tends to be higher in MMP-9 expression with score 0 and +1. Likewise, at other stages that did not show a tendency for higher clinical stages to express higher MMP-9. The results of the Spearman correlation test prove the statement above which shows that there is no significant relationship between MMP-9 expression and PTC clinical stage ($P > 0.05$). The results of the analysis based on the values of T, N, and M on MMP-9 also found that there were no statistically significant association ($P > 0.05$ for all).

Table 3. Association between clinical staging and tumor status with MMP-9 expression

Parameter	MMP-9 expression								Total	r	P value	
	0		+1		+2		+3					
	n	%	n	%	n	%	n	%				
Clinical stage												
I	5	31.3%	6	37.5%	3	18.8%	2	12.5%	16	100%	0.28	0.16
II	2	40.0%	1	20.0%	1	20.0%	1	20.0%	5	100%		
III	0	0.0%	2	100%	0	0.0%	0	0.0%	2	100%		
IVA	0	0.0%	0	0.0%	0	0.0%	1	100%	1	100%		
IVB	0	0.0%	0	0.0%	1	50.0%	1	50.0%	2	100%		
Total	7	26.9%	9	34.6%	5	19.2%	5	19.2%	26	100%		
Stage T												
T2	4	33.3%	4	33.3%	2	16.7%	2	16.7%	12	100%	0.23	0.26
T3	2	40.0%	1	20.0%	2	40.0%	0	0.0%	5	100%		
T3a	0	0.0%	1	100%	0	0.0%	0	0.0%	1	100%		
T4	0	0.0%	1	50.0%	0	0.0%	1	50.0%	2	100%		
T4a	1	25.0%	2	50.0%	1	25.0%	0	0.0%	4	100%		
T4b	0	0.0%	0	0.0%	0	0.0%	2	100%	2	100%		
Total	7	26.9%	9	34.6%	5	19.2%	5	19.2%	26	100%		
N												
N0	4	30.8%	4	30.8%	2	15.4%	3	23.1%	13	100%	0.1	0.62
N1	2	66.7%	1	33.3%	0	0.0%	0	0.0%	3	100%		
N1a	0	0.0%	1	50.0%	0	0.0%	1	50.0%	2	100%		
N1b	1	12.5%	3	37.5%	3	37.5%	1	12.5%	8	100%		
Total	7	26.9%	9	34.6%	5	19.2%	5	19.2%	26	100%		
M												

M0	5	22.7%	9	40.9%	4	18.2%	4	18.2%	22	100%		
M1	2	50.0%	0	0.0%	1	25.0%	1	25.0%	4	100%	-0.04	0.83
Total	7	26.9%	9	34.6%	5	19.2%	5	19.2%	26	100%		

Discussion

The results showed that the female was the predominant sex in this study. This result was in concordance with previous studies. Studies from several countries show that PTC was more common in female than in male. Gajowiec et al. in 2021 investigated whether sex can be used as a prognostic factor in PTC patients (13). The study used a retrospective approach by analyzing a total of 1,547 patients with PTC and reported that PTC was more common in female (female: 87.8% vs. male: 12.2%) (13–17). These studies supported the fact that PTC was more common in female patients. Based on age, we found in this study that PTC prevalence was higher in patients aged ≤ 55 years. Previous study reported that PTC was found mostly in patients aged 51–60 years and the prevalence was the lowest in patients aged less than 20 years (18). The high prevalence of patients aged ≤ 55 years might be caused by the low number of samples in this study.

In this study, we found that there was no significant relationship between MMP-9 expression and the clinical stage of PTC. Clinical staging based on the TNM system, which consisted of three aspects: size, nodule, and metastases). The higher stage is associated with higher chance of recurrence. The MMP-9 has physiological and pathological roles in inflammatory and neoplastic cells. Various components such as cytokines, lipopolysaccharides, TNF- α , extracellular matrix components, and growth factors can induce MMP-9 expression (8,9). Several studies have analyzed the relationship between MMP-9 and PTC. A study conducted by Armeriyanti, Mulyadi, and Maker in 2017 found that high MMP-9 expression might influence the extent of PTC infiltration and the expression of MMP-9 was associated with the aggressiveness of papillary thyroid carcinoma (19).

Several studies that have been done previously showed that MMP-9 can be used to assess the aggressiveness of papillary thyroid carcinoma, a predictor of tumor malignancy, and a tool for PTC diagnosis and prognostic prediction. A study by Heriyawati and Susilo showed that the difference in MMP-9 expression played a role in differentiating non-neoplastic thyroid nodule lesions from malignant neoplastic, but could not differentiate non-neoplastic thyroid nodule lesions from benign neoplastic lesions (20). In that study, MMP-9 was used as a potential predictor of tumor malignancy. Liu *et al.* found that the immunohistochemical expression of MMP-9 was markedly increased in PTC (17). Increased expression is associated with metastasis, advanced TNM stage and shorter patient survival. Assessment of MMP-9 expression in thyroid carcinoma samples may be a tool for PTC diagnosis and prognostic prediction. The study of Selemetjev *et al.* showed that VEGF-C and MMP-9 might serve as potential predictive biomarkers of aggressive PTC (12).

The clinical stage itself is related to the suspected occurrence of recurrence. Provisional conjectures made by researchers regarding the relationship between clinical stage and MMP-9 were based on scientific reasons for the role of MMP-9 in the process of invasion and metastasis. However, our result showed that there was no significant association between PTN stage and MMP-9 expression. However, our analysis showed that there was a positive correlation coefficient between MMP-9 expression level and staging, suggested that there was positive association between these variables.

There were limitations in this study. First, the relatively low number of subjects in this study. The low number of samples could be the reason for the absence of a significant association between the variables analyzed in this study. Further study with higher number of samples may be needed to be performed in the future. Second, the immunohistochemistry was only performed on one paraffin block. The immunohistochemistry examination performed in more than one paraffin block made from more than one tissue sample might be able to give more accurate result for MMP-9 expression determination.

Conclusion

There was no significant association between MMP-9 expression and clinical staging of papillary thyroid carcinoma.

Conflicts of interest

No competing interests declared.

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Authors' Contribution

T.M.S., D.F., and M.D.W. conceptualized, interpreted, analyzed, and wrote the manuscript. D.F., and M.D.W. reviewed the manuscript, supervised the study, and involved in the case management. All authors read and agreed to published version of the manuscript.

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